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Measles DNA vaccine priming for young infants

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Abstract

Despite the overall progress achieved with mass immunization campaigns in sub-Saharan Africa, measles mortality in young children remains a significant public health problem. Investigators at the Center for Vaccine Development (CVD) developed two Sindbis replicon-based measles DNA vaccine candidates encoding the measles virus (MV) hemagglutinin (H) or H and fusion (F) proteins to specifically target infants who are too young to receive the currently licensed measles vaccines. The Sindbis DNA replicons were well tolerated and highly immunogenic, eliciting plaque reduction neutralizing antibodies and measles-specific IFN-γ secreting T cells when administered to cotton rats, newborn and adult mice, and to juvenile and very young infant rhesus monkeys. A heterologous prime-boost regimen consisting of parenteral priming with DNA vaccine encoding H (pMSIN-H) and boosting with aerosolized attenuated MV vaccine was well tolerated by very young infant rhesus macaque monkeys and protected against viremia following respiratory challenge with wild type MV. A randomized, double-blind, placebo-controlled, Phase 1 clinical trial was conducted to evaluate the safety of these DNA vaccines in healthy adults living in the U.S.A. Three dosage levels of ~200, 400 and 800 μg of each DNA vaccine administered in a 2-dose regimen were found to be safe and well tolerated. Among the various candidate DNA vaccine strategies for young infants, this is the most advanced, having been tested in a Phase 1 clinical study. If successful, the proposed strategy would allow to prevent the "window of vulnerability" that otherwise opens at ~16 weeks of age as maternal antibodies wane.

Keywords: *Measles; Young infants; DNA vaccines; Prime-boost.*

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1. Introduction

In 1999, the World Health Organization (WHO) reported that measles was the third most important cause of mortality among children less than 5 years of age in developing countries [1], with most deaths occurring among children living in certain areas of the Indian sub-continent and in sub-Saharan Africa [2]. Disease burden persisted despite the existence of highly efficacious attenuated measles vaccines recommended to be given routinely through the Expanded Program on Immunization (EPI) to infants ~ 9 months of age living in developing countries.

A limitation of the live attenuated measles vaccine (LAV) is that it fails to reliably immunize infants younger than 6 months of age. A notable proportion of measles deaths in developing countries occurs during the so-called "window of vulnerability", which spans approximately 4 to 9 months of age [3]. During this period, antibodies of maternal origin drop to a level that cannot provide protection against clinical infection [4] but that can still interfere with successful immunization using the currently available LAV [5,6]. The immaturity of the immune system has also been implicated in the limited responses to measles immunization during the first months of life [7]. Consequently, severe clinical disease can ensue when young infants are exposed to wild type virus [8,9]. Considerable progress has been made since 2000 in diminishing measles mortality by improving routine vaccination and implementing a second opportunity for immunization through national and sub-national mass immunization campaigns [10,11]. Young infants are indirectly protected if mass campaigns achieve high levels of coverage [~90%], which diminishes the transmission of wild type MV in the community. However, maintaining high levels of coverage remains difficult [12]. An estimated 164 000 deaths from measles still occurred in 2008 [10] and a persistent high case fatality rate among children under 5 years of age with poor access to appropriate health care has been reported in several countries in sub-Saharan Africa [13], with infants younger than 12 months of age being most affected. Currently, the herd immunity that results from high coverage rates among individuals 10 months of age and older is the only mechanism available for protecting young infants. Thus, a safe and practical means to directly protect infants who are too young to respond reliably to the currently licensed measles vaccines would represent a useful adjunct tool for measles control.

2. Protection of young infants against measles

Several approaches have been assessed for immunizing young infants during the window of vulnerability. One strategy evaluated a 100-fold higher than usual dose of vaccine [14]. A second approach explored specific strains of attenuated MV (e.g., Edmonston Zagreb (EZ) strain) [15,16]. The third strategy involved aerosol administration of LAV [17]. The first strategy was abandoned due to safety concerns after clinical trials in several developing countries showed a poorly understood but significant increase in overall mortality among girls who received the high dose compared with the standard dose of vaccine [14]. The second approach was discarded when no specific attenuated strain proved to be markedly superior to other currently licensed strains [16]. The aerosol approach has shown some promise for older infants and children, especially for boosting vaccination (reviewed in [18]). But in young infants this approach has been compromised by the lack of a practical and efficient method of administering aerosolized vaccine and by inconsistent results [17] which sometimes revealed lower immunogenicity compared with subcutaneous immunization [19-21].

Herd immunity has been invoked as a means of protecting young infants during the window of vulnerability. As immunization coverage increases in a community, the risk of measles exposure is expected to diminish. However, this strategy has not always been effective. The licensed vaccine has a 95% seroconversion rate when given at the age of 12 months [16] and immunity in the population has to be over 95% to prevent endemic measles transmission [22]. If crowding is a factor, susceptible individuals can become infected even if vaccine coverage is high [23]. In urban districts of Guinea-Bissau, an increase in vaccine coverage from 61% to 80% did not reduce measles incidence among infants < 9 months of age, presumably due to the virus' extreme contagiousness [24]. Furthermore, sustained control of endemic measles requires a first dose of vaccine at 9-12 months plus a second dose provided either through routine services and/or repeated supplemental campaigns [11]. The occurrence of multi-country outbreaks involving tens of thousands of cases in Latin America (following an importation) illustrates the daunting task of sustaining measles elimination despite the implementation of supplemental campaigns [25].

Currently, all countries in the Americas and selected countries in Europe, the Middle East, sub-Saharan Africa, Oceania, and Asia have adopted immunization strategies aimed at measles elimination and have made substantial progress towards this goal [26,27,10]. These successes have led to believe that measles elimination in all regions of the world is feasible on the basis of existing measles vaccination strategies [28]. However, some authorities remain skeptical, taking into account the extreme transmissibility of measles and the limitations of the current vaccines [29]. Still others take the view that an improved vaccine that could reliably immunize and protect very young infants might be needed to eliminate measles in regions such as sub-Saharan Africa [30].

3. Immunological correlates of protection

Humoral immunity is important to prevent viral entry into cells that could initiate infection. This is clearly shown by the protection conferred to newborns by maternal antibodies and the efficacy of post-exposure administration of measles immune globulins to susceptible individuals (reviewed in [31]). The strongest correlate of protection against measles is the presence of plaque reduction neutralization (PRN) serum antibodies, of which a titer > 1:120 (or > 120-200 mIU/ml) has been associated with clinical protection [32]. Studies in rhesus macaques have shown that high avidity neutralizing antibodies are required to avoid occurrence of an enhanced disease, also known as atypical measles syndrome, as was seen in recipients of formalin-inactivated measles vaccine (reviewed in [31]). Cell mediated immunity (CMI), particularly $CD8^+$ cytotoxic T lymphocytes (CTLs), appears to play a critical role in recovery from illness by controlling viral replication and dissemination [33,34]. Agammaglobulinemic patients can recover normally from measles, indicating that CMI alone can be effective in the absence of antibodies [35]. In contrast, mortality in patients with T cell deficiencies (e.g., HIV) can reach 50-100% [36]. The contribution of CMI in the prevention of measles infection has been increasingly recognized in the light of "failed" seroconversion after vaccination [37-39]. Measles has long been associated with immunosuppression characterized by inhibition of T cell proliferation, impaired antigen presentation and cytotoxic function, reduced B lymphocyte maturation and antibody production and switch from Th1 to Th2 type cytokine polarization [40,41]. The mechanisms underlying these effects are still poorly understood.

4. Measles DNA vaccine priming of very young infants

Because of their capacity to produce vaccine antigens in an intracellular niche, DNA vaccines offer a promising means of priming young infants in the face of placentally transferred maternal antibodies [42,43]. In newborn mice, DNA vaccines elicit high quality and long-lasting antibody responses and can overcome deficient induction of Th1 and CTL responses, enhancing the capacity of young hosts to clear intracellular pathogens [44,45,46,47]. DNA vaccines have been used in combination with other vaccine delivery systems in 'heterologous prime-boost strategies' to enhance protective immunity against infectious agents, particularly viruses and protozoa [48]. The prime-boost approach increases and broadens immune responses compared with a single immunization or a homologous prime-boost, demonstrated by higher antibody levels and frequency of antigen-specific T cells, selective enrichment of high avidity antibodies and T cells, and increased efficacy against pathogen challenge [48,49]. Prime-boost immunization has gained increased attention as a practical and effective means to stimulate immune responses at very early life stages [49-51].

5. New Sindbis-based replicon measles DNA vaccines.

CVD investigators developed two Sindbis replicon-based measles DNA vaccine candidates to specifically target infants who are too young to receive the currently licensed measles vaccine [52-56]. The aim of these measles DNA

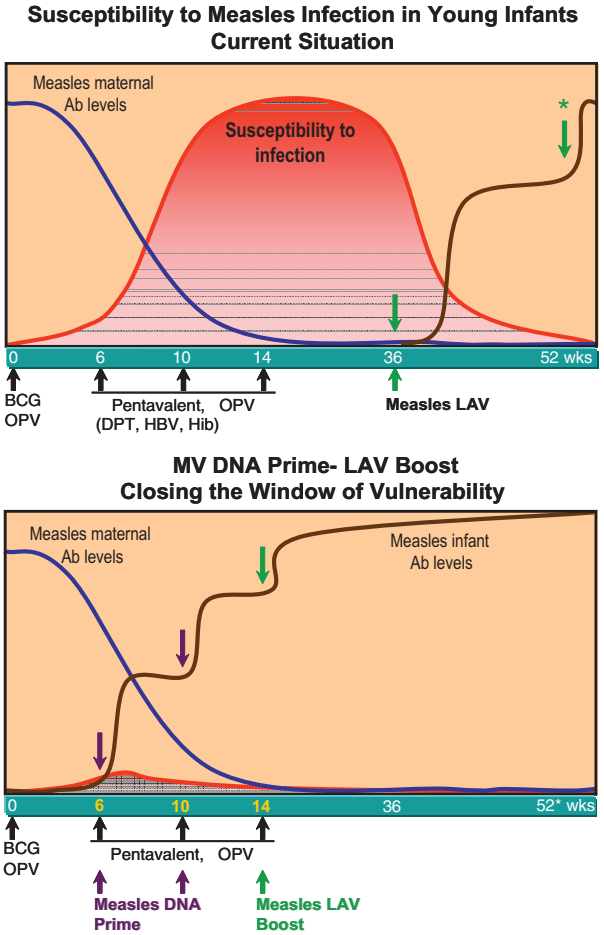

Figure 1. Measles prime-boost immunization strategy to close the window of vulnerability to infection. *Top*, Current situation; measles eradication strategies aim to achieve and maintain high coverage with 2 doses of measles vaccine to children aged 9 months to ≤20 years (*). *Bottom*, Proposed prime-boost immunization strategy. A Sindbis-based MV DNA vaccine could be given to infants at 6 and 10 weeks of age as priming immunogen, followed by live attenuated measles vaccine (LAV) at 14 weeks. Ab, antibody;BCG: Bacillus Calmette-Guerin; pentavalent: DPT (diptheria toxoid, pertussis, and tetatus toxoid), HBV (hepatitis B vaccine) and Hib (*Haemophilus influenzae* type b vaccine); OPV: oral polio vaccine.

vaccines is to prime the young infant immune system to respond safely and effectively to a subsequent boost with the currently licensed attenuated measles vaccine (**Figure 1**).

Sindbis replicons represent a new generation of improved DNA vaccines in which cDNAs driven by eukaryotic promoters express self-replicating replicon RNAs [57]. Transcription from the cytomegalovirus promoter within a mammalian cell gives rise to a Sindbis virus RNA replicon vector that programs its own cytoplasmic RNA amplification and high level expression of the heterologous measles gene(s) via the alphavirus subgenomic promoter. The increased immunogenicity of Sindbis-based DNA vaccines is due, in part, to increased antigen production. In addition, cells transfected with Sindbis DNA replicons elaborate double-stranded RNA, which enhances immune responses by stimulating Toll-like receptor 3 on antigen presenting cells and induces various cytokines. Cells transfected with Sindbis virus-based plasmids undergo apoptotic death, releasing antigenic material for cross-presentation and dsRNA that provides additional pro-inflammatory immune stimulation [58,59]. Furthermore, Sindbis virus-derived dsRNA can activate and enhance maturation of dendritic cells [60], a major requirement for the induction of Th1-type immunity early in life [61].

A modified backbone Sindbis replicon (pSINCP) developed by scientists at Novartis, which incorporates nonstructural protein gene sequences from a human dendritic cell-tropic Sindbis virus, was used as a vector to carry the genes that encode the measles hemagglutinin (H) antigen and the measles fusion (F) protein [52,56]. The H protein mediates viral entry into the host cell and is the main viral antigen against which neutralizing antibodies are directed. The F protein mediates fusion of the viral envelope with the cell membrane. CMI responses are typically broader if both proteins are included in the vaccine [56]. It was believed that the safety of the vaccine would be enhanced if both F and H antigens were included [62]. The imbalance in antibodies due to the absence of F was thought to be responsible for the atypical measles syndrome seen in the 1960s in children who received the formalin-inactivated measles vaccine [63]. Robust data do not exist to support this notion and, indeed, recent data generated in the rhesus challenge model refute the view [64]. Thus, Polack et al. showed that juvenile monkeys immunized with measles DNA vaccines encoding H and F proteins alone or in combination mounted protective PRN antibody titers and long-lasting $CD8^+$ CTLs and did not develop atypical measles after challenge with wild type MV [65]. Furthermore, if one wishes to deliver both H and F genes as part of a DNA vaccine, engineering a single DNA vaccine construct is preferable to co-administering two different plasmids in terms of diminishing the complexity, logistics, cost of manufacture and quality control of the product [56]. Two Sindbis replicons were produced: pMSIN-H, which contains only the H gene, and pMSINH-FdU, a bicistronic construct that contains both the H and the F genes [52,56].

6. Pre-clinical safety, immunogenicity, and protective efficacy

The Sindbis DNA replicons encoding MV antigens were administered to small animals (cotton rats and newborn and adult mice) intramuscularly (i.m.) [52,55,56,66], to very young infant (~ 45 days of age) rhesus monkeys intradermally (i.d.) by means of the Biojector® 2000 needle-free injection device [53] and to juvenile rhesus monkeys either i.d or i.m. The DNA vaccines were well tolerated in all the animal species tested. They were also immunogenic in adult and newborn mice and induced protective immunity against measles infection in cotton rats. Immune responses were further increased when a 2-dose DNA i.d. priming series was followed by i.m. boost with live attenuated EZ measles vaccine. In very young rhesus infants as well as in juvenile rhesus monkeys, the measles DNA vaccines elicited PRN antibodies and measles specific IFN-γ secreting T cells. In these immunogenicity studies in monkeys and in rodents, pMSIN-H stimulated stronger serum PRN responses than pMSINH-FdU. Priming with pMSIN-H succeeded in eliciting PRN titers above the protective threshold. pMSIN-H was also highly immunogenic in newborn mice in the presence of maternal antibodies, and the antibodies produced were of high avidity and neutralizing capacity. In very young infant macaques, 1.0 mg priming doses were more immunogenic than 0.5 mg doses. The i.d. route also proved somewhat more immunogenic for priming than the i.m. route.

A heterologous prime-boost regimen consisting of priming with 1.0 mg i.d. doses of pMSIN-H or pMSINH-FdU and boosting with aerosolized attenuated measles virus vaccine was well tolerated by juvenile macaques and protected against disease and viremia following challenge with wild type measles virus up to 16 months later. In very young infant rhesus macaque monkeys, a prime-boost regimen consisting of priming with 1.0 mg or 0.5 mg i.d. doses of pMSIN-H or 0.5 mg doses of pMSINH-FdU and boosting with aerosolized attenuated measles virus vaccine was well tolerated and protected against viremia following challenge with wild type measles virus 9 months later. No evidence was found in these experiments of histopathological features consistent with “atypical measles” [65, 67]. Future studies of interest for these vaccines in non-human primates include immunogenicity in the presence of maternal antibodies and analysis of immunosuppression.

7. Biodistribution, integration and toxicology

Studies assessing the biodistribution of the DNA vaccines were undertaken to detect any evidence of DNA integration [54]. pMSIN-H and pMSINH-FdU were administered i.d. to New Zealand White Rabbits at their intended clinical dosage levels via the Biojector 2000 injection system and biodistribution was monitored during a 60-day period. A single dose of 1.76 mg of pMSIN-H or 1.84 mg of pMSINH-FdU had no effect on mortality, clinical and cage-side observations, body weights, or food consumption. The only vaccine-related effects observed were minimal transient erythema, edema, and inflammation confined to the injection site. The plasmids persisted

for the duration of the study at the injection site in the skin and subcutis, or the muscle, and, to a much lesser degree, in the popliteal lymph nodes [54]. There was no evidence of plasmid integration into the rabbit host genome.

To assess potential toxicological effects, New Zealand White Rabbits were primed i.d. with pMSIN-H (1.76 mg), pMSINH-FdU (1.84 mg) or PBS. Some animals received a subcutaneous (s.c.) injection (boost) of 0.5 ml of PBS or ~10^3 tissue culture 50% infectious doses (TCID50) of the EZ measles vaccine. No effects were found on mortality, clinical and behavorial observations, body weights, food consumption, clinical pathology, or organ weights. Increased frequency, score, and recovery time of dermal Draize observations at the injection sites were observed, which correlated with gross and histopathological findings of inflammation that resolved with time [54]. Both Sindbis-based vaccine plasmids were immunogenic in rabbits, with pMSIN-H eliciting higher PRN titers.

8. Phase I clinical studies

The extensive pre-clinical data demonstrating the safety, immunogenicity and efficacy of the Sindbis replicon measles vaccines led to the filing of a New Investigational Drug Application to support the performance of a Phase I clinical trial. Based on the superior immunogenicity and efficacy of pMSIN-H in the pre-clinical experiments, it was the favored DNA vaccine candidate to move forward in clinical trials. Nevertheless, on the assumption that humans might respond differently, we elected also to study the pMSINH-FdU plasmid, at least in Phase I.

We conducted the Phase I trial of the DNA vaccines in healthy adults, aged 18-45 years, living in the U.S.A., who participated in a randomized, double-blind, placebo-controlled, dose-escalating, outpatient study to assess 3 dosage levels of approximately 200, 400 and 800 μg of each vaccine in a stepwise fashion. The vaccines were administered i.d. using Biojector 2000 (Kotloff & Levine, personal communication). Since routine infant EPI immunization involves contacts at 6, 10 and 14 weeks of age, the ultimate goal will be to administer a MV DNA-based vaccine at 6 and 10 weeks of age as the priming immunogen, followed by a dose of currently licensed attenuated measles vaccine as the boosting immunogen at 14 weeks of age (**Figure 1**). This strategy, if successful, would allow an infant to be immunized before the window of vulnerability opens at ~16 weeks of age.

9. Conclusion

Despite the overall progress achieved with mass immunization campaigns in several countries in sub-Saharan Africa and Asia, measles mortality in young children remains a serious health problem [13]. A Sindbis replicon measles DNA vaccine encoding the measles H protein was shown to be highly immunogenic and to induce protective immunity in non-human primates. This vaccine could be administered as priming immunogen at 6 and 10 weeks of age, followed by the currently licensed attenuated measles vaccine (possibly administered through aerosol) at 14 weeks of age. A Phase I study in humans showed that the H-encoding Sindbis replicon is well tolerated and immunogenic. The proposed strategy could provide a means of protecting infants during the critical window of vulnerability.

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